

FIGURE 1

Human G Protein Coupled Receptor Family
(Receptors known as of January, 1999)

CLASS	LIGAND	NUMBER	TISSUE	PHYSIOLOGY	THERAPEUTICS
•Class I Rhodopsin like					
	•Amine				
	•Acetylcholine (muscarinic & nicotinic)	5	Brain, Nerves, Heart	Neurotransmitter	Acuity, Alzheimer's
	•Adrenoceptors				
	•Alpha Adrenoceptors	6	Brain, Kidney, Lung	Gluconeogenesis	Diabetes, Cardiovascular
	•Beta Adrenoceptors	3	Kidney, Heart	Muscle Contraction	Cardiovascular, Respiratory
	•Dopamine	5	Brain, Kidney, GI	Neurotransmitter	Cardiovascular, Parkinson's
	•Histamine	2	Vascular, Heart, Brain	Vascular Permeability	Anti-inflammatory, Ulcers
	•Serotonin (5-HT)	16	Most Tissues	Neurotransmitter	Depression, Insomnia, Analgesic
	•Peptide				
	•Angiotensin	2	Vascular, Liver, Kidney	Vasoconstriction	Cardiovascular, Endocrine
	•Bradykinin	1	Liver, Blood	Vasodilation,	Anti-inflammatory, Asthma
	•C5a anaphylatoxin	1	Blood	Immune System	Anti-inflammatory
	•Fmet-leu-phe	3	Blood	Chemoattractant	Anti-inflammatory
	•Interleukin-8	1	Blood	Chemoattractant	Anti-inflammatory
	•Chemokine	6	Blood	Chemoattractant	Anti-inflammatory
	•Orexin	2	Brain	Fat Metabolism	Obesity
	•Nociceptin	1	Brain	Bronchodilator, Pain	Airway Diseases, Anesthetic
	•CCK (Gastrin)	2	Gastrointestinal	Motility, Fat Absorption	Gastrointestinal, Obesity, Parkinson's
	•Endothelin	2	Heart, Bronchus, Brain	Muscle Contraction	Cardiovascular, Respiratory
	•Melanocortin	5	Kidney, Brain	Metabolic Regulation	Anti-inflammatory, Analgesics
	•Neuropeptide Y	5	Nerves, Intestine, Blood	Neurotransmitter	Behavior, Memory, Cardiovascular
	•Neurotensin	1	Brain,	CNS	Cardiovascular, Analgesic
	•Opioid	3	Brain,	CNS	Depression, Analgesic
	•Somatostatin	5	Brain, Gastrointestinal	Neurotransmitter	Oncology, Alzheimer's
	•Tachykinin				
	(Substance P, NKA ₁)	3	Brain Nerves	Neurohormone	Depression, Analgesic

•Thrombin	3	Platelets, Blood Vessels	Coagulation	Anti-coagulant, Anti-inflammatory
•Vasopressin-like	4	Arteries, Heart, Bladder	Water Balance	Anti-diuretic, Diabetic Complications
•Galanin	1	Brain, Pancreas	Neurotransmitter	Analgesics, Alzheimer's
•Hormone protein				
•Follicle stimulating hormone	1	Ovary, Testis	Endocrine	Infertility
•Lutropin-choriogonadotropin	1	Ovary, Testis	Endocrine	Infertility
•Thyrotropin	1	Thyroid	Endocrine	Thyroidism, Metabolism
•(Rhod)opsin				
•Opsin	5	Eye	Photoreception	Ophthalmic Diseases
•Olfactory	4(~1000)	Nose	Smell	Olfactory Diseases
•Prostanoid				
•Prostaglandin	5	Arterial, Gastrointestinal	Vasodilation, Pain	Cardiovascular, Analgesic
•Lysophosphatidic Acid	2	Vessels, Heart, Lung	Inflammation	Cancer, Anti-Inflammatory
•Sphingosine-1-phosphate	2	Most Cells	Cell proliferation	Cancer
•Leukotriene	1	White Blood Cells, Bronchus	Inflammation	Asthma, Rheumatoid Arthritis
•Prostacyclin	1	Arterial, Gastrointestinal	Platelet Regulation	Cardiovascular
•Thromboxane	1	Arterial, Bronchus	Vasoconstriction	Cardiovascular, Respiratory
•Nucleotide-like				
•Adenosine	4	Vascular, Bronchus	Multiple Effects	Cardiovascular, Respiratory
•Purinoceptors	4	Vascular, Platelets	Relaxes Muscle	Cardiovascular, Respiratory
•Cannabis	2	Brain	Sensory Perception	Analgesics, Memory
•Platelet activating factor	1	Most Peripheral Tissues	Inflammation	Anti-inflammatory, Anti-asthmatic
•Gonadotropin-releasing hormone like				
•Gonadotropin-releasing hormone	1	Reproductive Organs, Pituitary	Reproduction	Prostate Cancer, Endometriosis
•Thyrotropin-releasing hormone	1	Pituitary, Brain	Thyroid Regulation	Metabolic Regulation
•Growth hormone- inhibiting factor	1	Gastrointestinal	Neuroendocrine	Oncology, Alzheimer's
•Melatonin	1	Brain, Eye, Pituitary	Neuroendocrine	Regulation of Circadian Cycle
•Secretin	1	Gastrointestinal, Heart	Digestion	Obesity, Gastrointestinal
•Calcitonin	1	Bone, Brain	Calcium Resorption	Osteoporosis
•Corticotropin releasing factor/urocortin	1	Adrenal, Vascular, Brain	Neuroendocrine	Stress, Mood, Obesity
•Gastric inhibitory peptide (GIP)	1	Adrenals, Fat Cells	Sugar/Fat Metabolism	Diabetes, Obesity
•Glucagon	1	Liver, Fat Cells, Heart	Gluconeogenesis	Cardiovascular

•Class II
Secretin like

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Peptide	Concentration	Target Organ	Effect	Reference
•Glucagon-like Peptide 1 (GLP-1)	1	Pancreas, Stomach, Lung	Gluconeogenesis	Cardiovascular, Diabetes, Obesity
•Growth hormone-releasing hormone	1	Brain	Neuroendocrine	
•Parathyroid hormone	1	Bone, Kidney	Calcium Regulation	
•PACAP	1	Brain, Pancreas, Adrenals	Metabolism	
•Vasoactive intestinal polypeptide (VIP)	1	Gastrointestinal	Motility	
•Metabotropic Glutamate	7	Brain	Sensory Perception	Hearing, Vision
•GABA _B	1	Brain	Neurotransmitter	
•Extracellular Calcium Sensing	1	Parathyroid, Kidney, GI Tract	Calcium Regulation	Cataracts, GI Tumors

●ClassIII

Figure 2

G protein-coupled receptors:

(Division into Class A

Or Class B)

1. **A1 adenosine receptor** [Homo sapiens]. ACCESSION AAB25533
npivyaf riqkfrvtfi kiwndhfrcq pappidedlp eerpdd
Class A
2. **adrenergic, alpha -1B-, receptor** [Homo sapiens]. ACCESSION NP_000670
npiitypc sskefkrafv rilgcqcrgr grrrrrrrr lggcaytyrp wtrggslers qsrkdsldds gscslsgsqr
lpsaspspgy lrggappve lcafepwkap gallslpape ppgrgrhds gplftfkilt epespgtdgg asnggceaaa
dvangppgfk snmplapgqf
Class A
3. **adrenergic receptor alpha-2A** [Homo sapiens]. ACCESSION AAG00447
npviytifn hdfirafkki lrgdrkriv
Class A
4. **alpha-2B-adrenergic receptor** - human. ACCESSION A37223
npviytifn qdfirafri lcrpwtqtaw
Class A
5. **alpha-2C-adrenergic receptor** - human. ACCESSION A31237
npviytfvfn qdfirpsfkh i lfrrrrgfr q
Class A
6. **beta-1-adrenergic receptor** [Homo sapiens]. ACCESSION NP_000675
npiiyers pdfrafqgl lccarraarr rhathgdrpr asgclarpgp ppspgaasdd ddddvvgatp parllepwag
cnggaaadsd ssldepcrpg faseskv
Class A
7. **beta-2 adrenergic receptor**. ACCESSION P07550
npliyersp dfriafqell clrrsslkay gngyssngnt 361 geqsgyhveq ekenkllced lpgtedfvgh qgtvpsdnid
sqgmcsnd sll
Class A
8. **dopamine receptor D1** [Homo sapiens]. ACCESSION NP_000785
npii yafnadfrka fstllgcyr lcpatnnaiet vsinnngaam fsshheprgs iskecnlvyl iphavgsed
lkkeaagia rpleklspal svldytdtv slekiqpitiq ngqhpt
Class A
9. **D(2) dopamine receptor**. ACCESSION P14416
npiiyttfn iefrakfli lhc
Class A

10. **d3 dopamine receptor** - human. ACCESSION G01977
np viyttfnief rkafkilsce
Class A
11. **dopamine receptor D4** - human. ACCESSION DYHUD4
npviyvtv fnaefrnvfr kalracc
Class A
12. **dopamine receptor D5** - human. ACCESSION DYHUD5
npviya fnadfqqkva qllgcshfcs rtpvetvnis nelisynqdi vfhkeiaaay ihmmpnavtp gnrevdndee
egpfdrmfqi yqtspdgdpv aesvweldce geislckitp ftpngfh
Class A
13. **muscarinic acetylcholine receptor M1** [Homo sapiens]. ACCESSION NP_000729
nrmcyal cnkafirdtfr llllcrwdkr rwrkipkrpg svhrtpsraq
Class A
14. **muscarinic acetylcholine receptor M2** [Homo sapiens]. ACCESSION NP_000730
npacy alcnatfkkt fkhllmchyk nigatr
Class A
15. **muscarinic acetylcholine receptor M3** [Homo sapiens]. ACCESSION NP_000731
n pvcyalcnkt firtfkmlll cqcdkkkrrk qqyqqrqsvi fhkrapeqal
Class A
16. **muscarinic acetylcholine receptor M4** [Homo sapiens]. ACCESSION NP_000732
npa cyalcnatfk ktrfhlllcq yrnigtar
Class A
17. **m5 muscarinic receptor**. locus HUMACHRM ACCESSION AAA51569
npicyalcnr tfrktfkmll lcrwkkkkve eklywqgnsk lp
Class A
18. **5-hydroxytryptamine (serotonin) receptor 1A** [Homo sapiens]. ACCESSION BAA90449
npviy ayfnkdfqna fkkiikckf
Class A
19. **5-hydroxytryptamine (serotonin) receptor 1B** [Homo sapiens]. ACCESSION BAA94455
npiiyt msnedfkqaf hklirfkets
Class A
20. **5-hydroxytryptamine (serotonin) receptor 1E** [Homo sapiens]. ACCESSION BAA94458
n pllytsfnd fklafkklir cre
Class A

21. **OLFACTORY RECEPTOR 6A1.** ACCESSION O95222
npiyclrmq evkralccil hlyqhqpdp kkgssrnv
Class A
22. **OLFACTORY RECEPTOR 2C1.** ACCESSION O95371
npliy tlrnmevkga lrrllgkgre vg
Class A
23. **angiotensin receptor 1 [Homo sapiens].** ACCESSION NP_033611
npl fygflgkfk ryflqllkyi ppkakshsnl **sfkmsflsyr** psdnv**ssst**k kpapcfeve
Class B
24. **angiotensin receptor 2 [Homo sapiens].** ACCESSION NP_000677
npflycf vgnrfqqklr svfrvpitwl qgkresmscr **ksssl**remet fvs
Class B
25. **interleukin 8 receptor beta (CXCR2) [Homo sapiens].** ACCESSION NM_001557
NPLIYAFIGQKFRHGLLKILAIHGLISKDSLKPDSRPSFVGSSSGHTSTTL
Class B
26. **cx3c chemokine receptor 1 (cx3cr1) (fractalkine receptor)**
ACCESSION P49238
np liyafagekf rrylyhlygk clavlcgrsv hvdf**sssesq** rsrhg**svlss** nftyhtsdgd allll
Class B
27. **neurotensin receptor - human.** ACCESSION S29506
n pilynlvsan frhiflatla clcpvwrrrr krpafsrkad **svssnhflss** natretly
Class B
28. **SUBSTANCE-P RECEPTOR (SPR) (NK-1 RECEPTOR) (NK-1R).** ACCESSION P25103
npiiyccldn rrlgfkhafr rccpfisagd yeglemkstr yltqgsvyk vsrlet**st**svvgaheeepe dgpkat**psl**
dlts**ncssrs** dsktmtesfs **fssn**vl
Class B
29. **vasopressin receptor type 2 [Homo sapiens].** ACCESSION AAD16444
npwiyasfss svsselrll ccargtrpps lgpqdesc**tt** **assslakdts s**
Class B
30. **thyrotropin-releasing hormone receptor - human.** ACCESSION JN0708
npviy nlmsqkfraa frklenckqk ptekpanysv alnysvikes dhf**st**elddi **tvt**dtly**sat** kvsfddtcla sevsfsqs
Class B
31. **oxytocin receptor - human.** ACCESSION A55493
npwiym lftghlfhel vqrflccsas ylkgrlget **saskksnsss** fvlshr**sssq** rscsqpsta
Class B

32. **neuromedin U receptor [Homo sapiens].** ACCESSION AAG24793
npvlyslmssrfretfqaclgacchrlprhsshslsrmttgstlcdvgsllgswvhplagndgpeaqqetdps
Class B
33. **gastrin receptor.** ACCESSION AAC37528
npivy cfmhrrfrqa cletcarcep rpprarpral pdedpptpsi aslsrlsytt isflgpg
Class B
34. **galanin receptor 3 [Homo sapiens].** ACCESSION 10879541
nplv yalashrfa rfrlwpcgr rrrhraral rrvrpassgp pgcpgdarps grllagggqg pepregpvhg geaargpe
Class A
35. **edg-1 - human.** ACCESSION A35300
npiiy tltnkemrra firimscke psdgsagkfk rpiagmefs rsksdnsshp 361 qkdegdnpet imssgnvnss s
Class A
36. **central cannabinoid receptor [Homo sapiens].** ACCESSION NP_057167
npiiyair skdlrhafis mipscegtaq pidnsmgdsd clikhannaa svhraaescl kstivkiakvi msvstdtsae al
Class A
37. **delta opioid receptor - human.** ACCESSION I38532
npvlyaf ldenfkrcfr qlcrkpcgrp dpssfsrpre atarervtac tpsdpggggr aa
Class A
38. **proteinase activated receptor 2 (PAR-2) human.** ACCESSION P55085
dpfvyyfvshdfrdhaknallersvrtvkqmqvsltskkhsrksssyssssttvktsy
Class A
39. **vasopressive intestinal peptide receptor (VIPR) rat.** ACCESSION NM_012685
NGEVQAELRRKWRRWHLQGVLGWSSKSQHPWGGSNGATCSTQVSMLTRVSPSARR
SSSFQAEVSLV
Class B

Figure 3

A. Human V2R DNA (nucleotides encoding the last 29 amino acids of the V2R and the adjacent stop codon):

gcccggggacgcacccccaccagcctgggtccccaagatgagtcctgcaccaccgccagctcct
ccctggccaaggacacttcacgtga

B. PCR amplified human V2R DNA fragment:

gcggccgcacggggacgcacccccaccagcctgggtccccaagatgagtcctgcaccaccgcc
agctcctccctggccaaggacacttcacgtgaagatctccgcgtctaga

*Additions and changes to the V2R DNA are underlined.

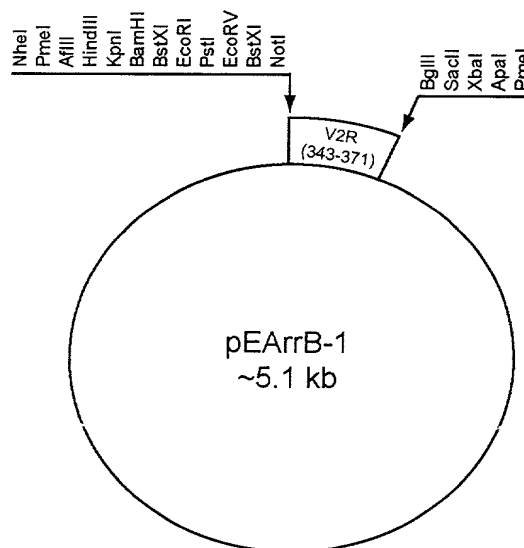
*The Sma I (cccggg) restriction enzyme site (underlined in Fig. 3A) was eliminated in the amplified DNA fragment by changing a cytosine to an adenine.

*A Not I restriction site (gcggccgc) was incorporated into the amplified DNA fragment by adding 6 nucleotides (gcggcc) to the 5' end of the V2R DNA.

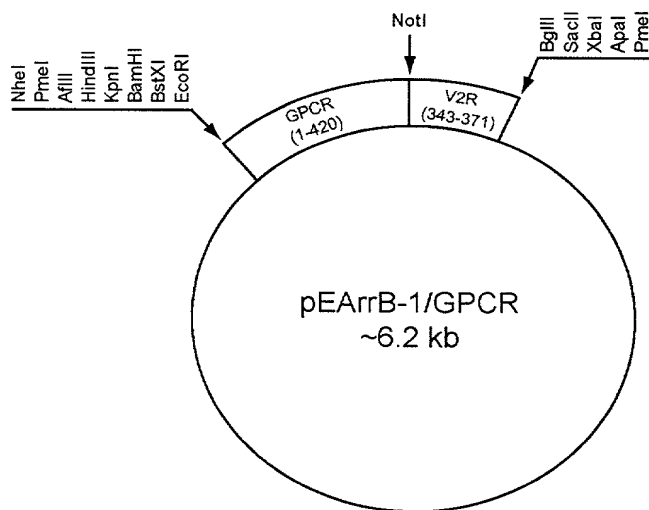
*Bgl II (agatct), Sac II (ccgcgg), and Xba I (tctaga) restriction enzyme sites were added to the 3' end of the V2R DNA.

Figure 4

A.



B.



C.

...AAARGRTPPSLGPQDESCCTASSSLAKDTSS

Figure 5

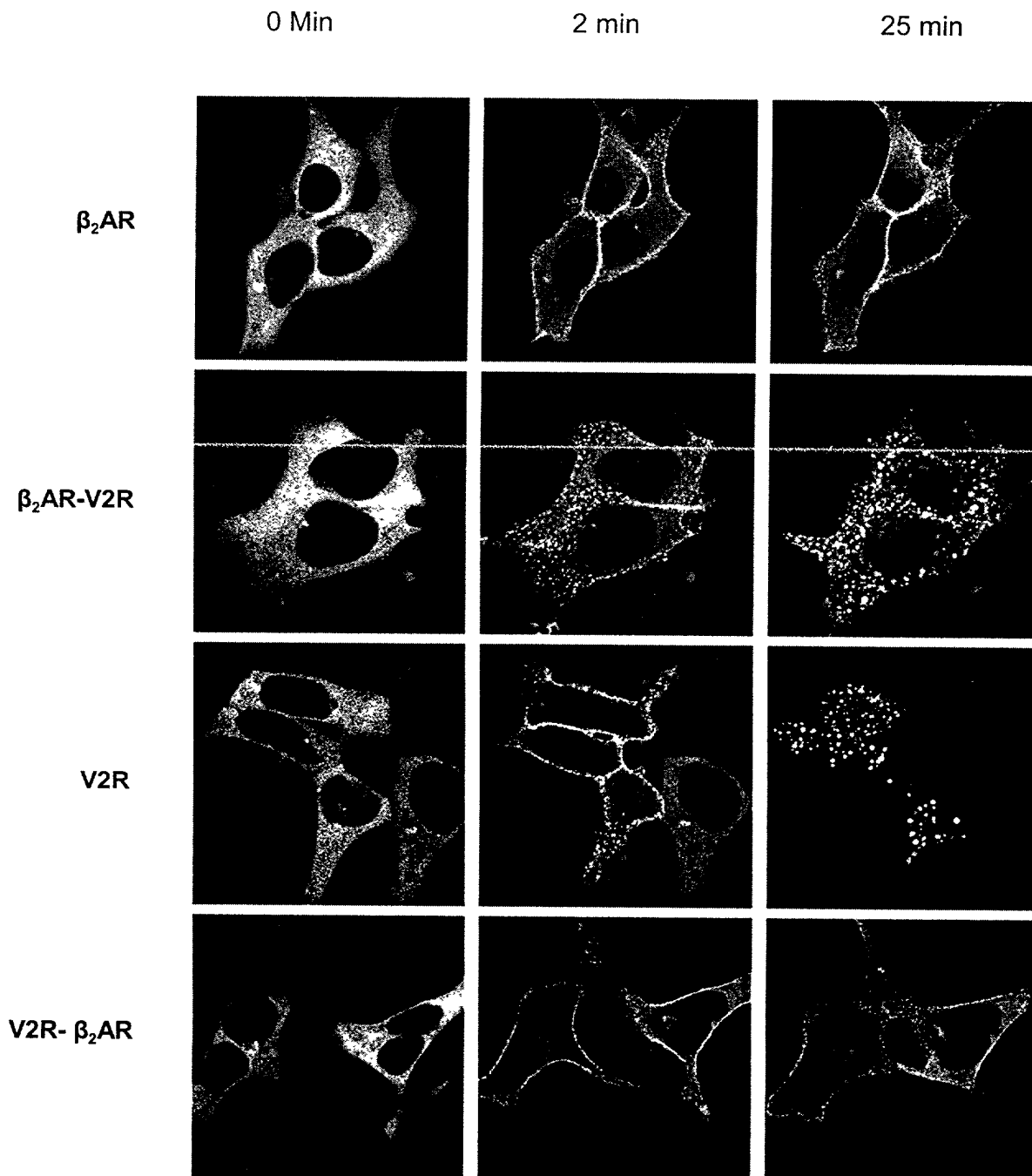


Figure 6

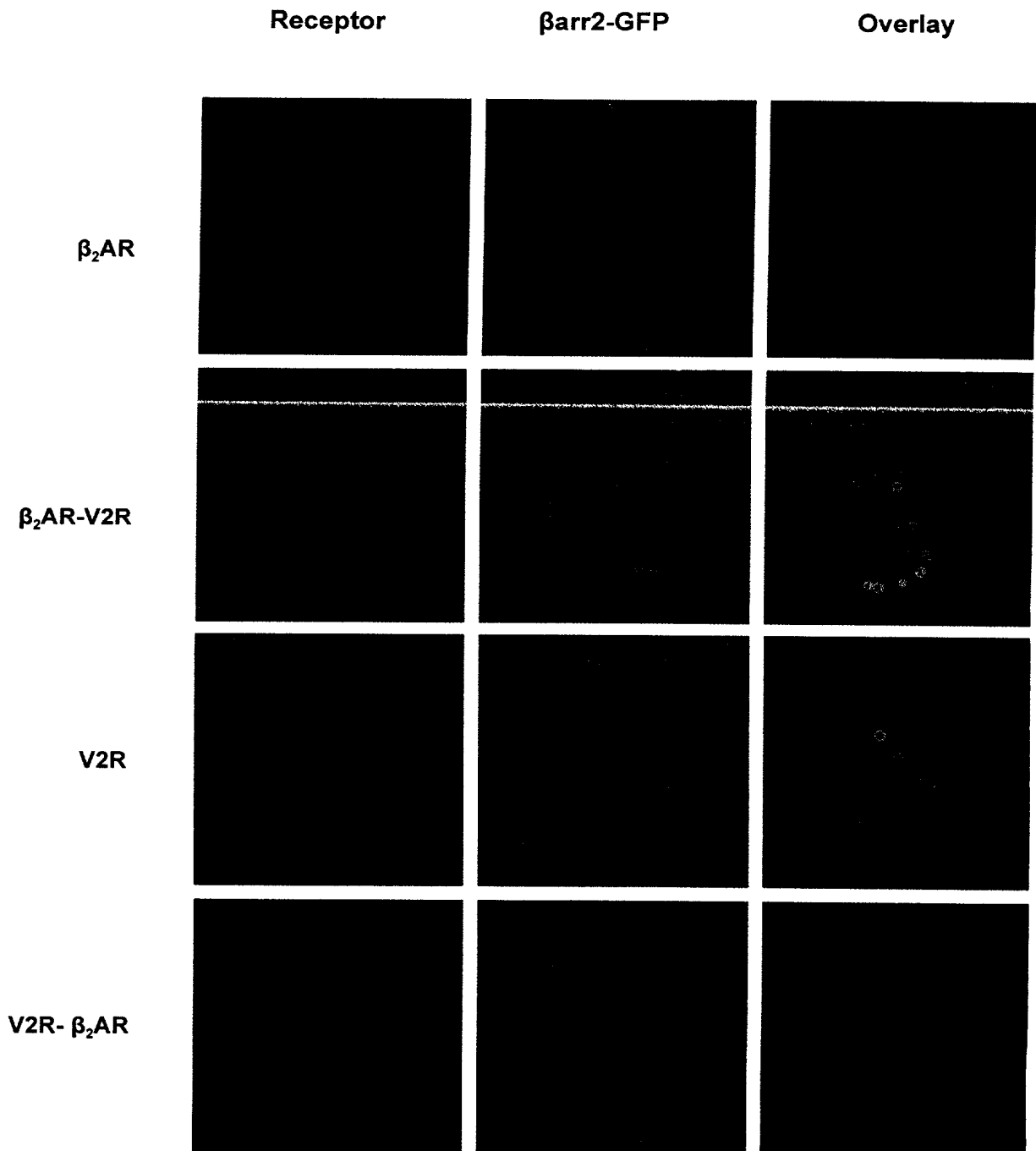


Figure 7

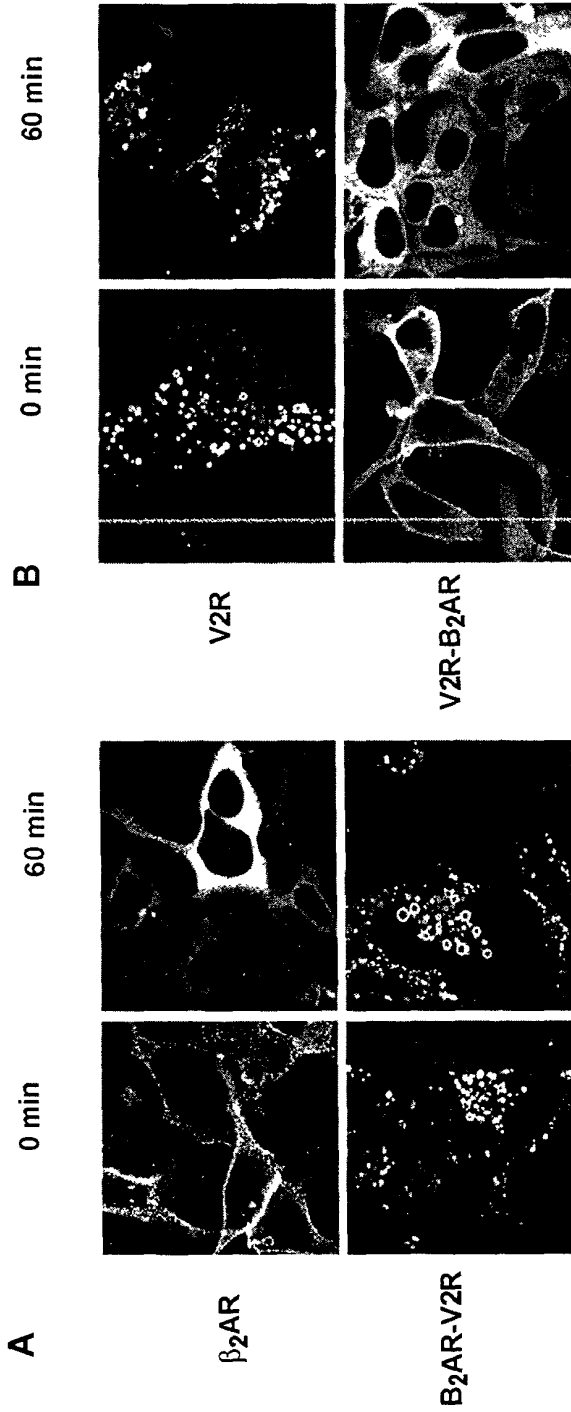


Figure 8

A

1) V2R	CARGRTPPSLGPQDESCCTTASSSLAKDTSS
2) V2R-S362X	CARGRTPPSLGPQDESCCTTA
3) V2R-SSSTSS/AAAAAA	CARGRTPPSLGPQDESCCTTAA AA ALAKD AAA
4) V2R-TSS/AAA	CARGRTPPSLGPQDESCCTTASSSLAKD AAA
5) V24-SSS/AAA	CARGRTPPSLGPQDESCCTTAAAAALAKDTSS
6) β_2 AR-V2R-SSS/AAA	CARGRTPPSLGPQDESCCTTAAAAALAKDTSS
7) β_2 AR	CLRRSSLKAYNGYSSNGNTGEQSGYHVEQEKENKLLCEDLP- GTEDFVGHQGTVPDNDIDSQGRNCSTNDLSLL
8) β_2 AR413-V2R10	CLRRSSLKAYNGYSSNGNTGEQSGYHVEQEKENKLLCEDLP- GTEDFVGHQGTVPDNDIDSQGRNCSTNDLSLL SSSLAKDTSS
9) β_2 AR360-V2R10	CLRRSSLKAYNGYSSNGNT SSSLAKDTSS

B

V2R	NPWIYASFSSVSSELRLSLLCCARGRTPPSLGPQDESCCTTASSSLAKDTSS
AAA-1	-----AAA-----
AAA-2	-----AAA-----
NTR-1	NPILYNLVSANFRQVFLSTLACLCPGWRHRRKKRPTFSRKPNSMSSNHAFSTSATRETTY
AMAA	-----A-AA-----
AAA	-----AAA-----
OTR	NPWIYMLFTGHLFHELIVQRFLLCCSAYLKGRRLLGETSASKNSSSFVLSHRSSSQRSCSQPSTA
AAAA	-----AAAA-----
AAA-1	-----AAA-----
AAA-2	-----AAA-----

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C

SPR	NPIIYCCINDRFRLGFKHAFRCPPFISAGDYEGLMKSTRYLQTVGVYKVRLETTISVVGAHEEEEPEDGPKATPSSLKLTNSCSRSDSKTMTESFSSNVLS
383X	-----X-----
355X	-----X-----
325X	-----X-----
AAIAA	-----AA-AA-----
APAA	-----A-AA-----

Figure 9

Amino Acid Sequence of the Wild-Type Receptors

A. Amino acid sequence of the wild-type V2R

MLMASTTSAPVPGHPSLPSLPSNSSQERPLDTRDPLLARAELALLSIVFVAVALSNGLVLAALARRGRRGHWAPIHVFIGHLCLADLAVALFQVLPQLAWKATDRFRGPDALCRAVKYLQMVGMYASSYMILAMTLDHRHAICRPMLAYRHGSGAHWNRPVLVAVAFSLLSLPQLFI FAQRNVEGSGSVTDCWACFAEPWGRRTYVTWIALMV FVAPT LGIAACQVLI FREI HASLVPGP SERP GRRRRGRRTGSPGEGAHVSAAVAKTVRMTLVIVVYVLCWAPFFLVQLWAAWDPEAPLEGA PFVLLMLLASLNSCTNPWIIYASFSSSVSELRSLLCC**CARGRTPPSLGPQDESCTTASSSLAKDTSS**

(Seq. ID No. 1)

B. Amino acid sequence of the wild-type β_2 AR

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMI VMSLIVLAIVFGNVLVITAI AKFERLQTVTNYFITSLACADLMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIE TLCVIAVDRYFAITSPFKYQSLLTKNKARV IILMVWIVSGLTSFLPIQMHWYRATHQEAIN CYANETCCDFFTQAYAIASSIVSFYVPLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQN LSQVEQDGRGTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQDNLIRK EVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSGY HVEQEKENKLLCEDLPGTEDFVGHQGTVPDNDISQGRNCSTNDSLL

(Seq. ID No. 2)

Amino Acid Sequence of the Chimeric Receptors

C. Amino acid sequence of the β_2 AR-V2R chimera (Oakley et al.)

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMI VMSLIVLAIVFGNVLVITAI AKFERLQTVTNYFITSLACADLMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIE TLCVIAVDRYFAITSPFKYQSLLTKNKARV IILMVWIVSGLTSFLPIQMHWYRATHQEAIN CYANETCCDFFTQAYAIASSIVSFYVPLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQN LSQVEQDGRGTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQDNLIRK EVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELL**CARGRTPPSLGPQDESCTTASSSLAKDTSS**

(Seq. ID No. 3)

*shown in bold are the amino acids that were moved to the β_2 AR to increase its affinity for arrestin.

Figure 10

A. Amino acid sequence of the MOR-V2R chimera expressed from the pEArrB-1/MOR vector

MDSSTGPGNTSDCSDPLAQASCSPAPGSWLNLSHVDGNQSDPCGLNRTGLG
GNDSLCPQTGSPSMVTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTA
TNIYIFNLALADALATSTLPFQSVNYLMGTWPFGTILCKIVISIDYYNMFT
SIFTLCTMSVDRIYAVCHPVKALDFRTPRNAKIVNVCNWILSSAIGLPVMF
MATTKYRQGSIDCTLTFSSHPTWYWENLLKICVFIFAFIMPILIIITVCYGLM
ILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVFIVCWTPIHIIYVIIKALI
TIPETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENFKRCFREFC**AAARGR**
TPPSLGPQDESCCTASSSLAKDTSS

(Seq. ID No. 4)

B. Amino acid sequence of the D1AR-V2R chimera expressed from the pEArrB-1/D1AR vector

MAPNTSTMDEAGLPAERDFSFRILTACFLSLLILSTLLGNTLVCAAVIRFR
HLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAGFWPFGSFCNIWVAFD
IMCSTASILNLCVISVDRIYWAISSPFQYERKMTPKAAFILISVAWTLVLI
SFIPVQLSWHKAKPTWPLDGNFTSLEDTEDDNCDTRLRSRTYAISSSLISFY
IPVAIMIVTYTSIYRIAQKQIRRISALERAAVHAKNCQTTAGNGNPVECAQ
SESSFKMSFKRETKVLKTLVIMGVFVCCWLPFFISNCMVFPFCGSEETQPF
CIDSITFDVFVWFGWANSSLNPIIYAFNADFQKAFSTLLGCYRLC**AAARGR**
TPPSLGPQDESCCTASSSLAKDTSS

(Seq. ID No. 5)

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C. Amino acid sequence of the 5HT1AR-V2R chimera expressed from the pEArrB-1/5HT1AR vector

MDVLSPGQGNNNTTSPPAPFETGGNTTGISDVTVSYQVITSLLLGTLI FCAV
LGNACVVAALALERSLQNVANYLIGSLAVTDLMSVSVLVLPMAALYQVLNKW
TLGQVTCDLFIALDVLCTSSILHLCAIALDRYWAITDPIDYVNRTPRRA
AALISLTWLIGFLISIPMLGWRTPEDRSDPDACTISKDHGYTIYSTFGAF
YIPLLLMLVLYGRIFRAARFRIRKTVKKVEKTGADTRHGASPAQPKKSVN
GESGSRNWRLGVESKAGGALCANGAVRQGGDALEVI EVHRVGNSKEHLP
LPSEAGPTPCAPASFERKNERNAEAKRKMALARERKTVKTLGIIMGTFILC
WLPFFIVALVLPFCESSCHMPTLLGAI
INWLGYSNSLLNPVIYAYFNKDFQNAFKKI IKCNFCAAARGRTPPSLGPQD
ESCTTASSSLAKDTSS

(Seq. ID No. 6)

D. Amino acid sequence of the β 3AR-V2R chimera expressed from the pEArrB-1/ β 3AR vector

MAPWPHENSSLAPWPDLP TLAPNTANTSGLPGPVWEAALAGALLALAVLAT
VGGNLLVIVAIWTPRLQTMNVFVTS LAAADLVMGLLVVPPAATLALTGH
WPLGATGCELWTSVDVLCVTAS IETLCALAVDRYLAVTNPLRYGALVTKRC
ARTAVVLVWVVSAAVSFAPIMSQWVRVGADAE AQRCHSNPRCCAFASNMPY
VLLSSSVSFYLP LLVMLFVYARV FVVATRQLRLLRGELGRFPPEESPPAPS
RSLAPAPVGT CAPPEGVPACGRPARLLPLREHRALCTLGLIMGTFTLCWL
PFFLANVLRALGGPSLVPGPAFLALNWLGYANS AFNPLIYCRSPDFRSAFR
RLLCRC **AAARGRTPPSLGPQDESCTTASSSLAKDTSS**

(Seq. ID No. 7)

E. Amino acid sequence of the Edg1R-V2R chimera expressed from the pEArrB-1/Edg1R vector

MGPTSVPLVKAHRSSVSDYVNYDI IVRHNYTGKLNISADKENS IKLTSV
FILICCFIILENIFVLLTIWKT KKFHRPMYYFIGNLALS DLLAGVAYTANL
LLSGATTYKLTPAQWFLREGSMFVALSASVFSLLAIAIER YITMLKMKLHN
GSNNFRLFLLISACWVISLILGGLPIMGWNCISALSSCSTVLPLYHKHYIL
FCTTVFTLLLLSIVILYCRIYSLVRTRSRLTFRKNISKASRSSEKSLALL
KTVIIVLSVFIACWAPLFILLLLDVGCKVKTC DILFRAEYFLVLAVLNSGT
NP I IYTLTNKEMRRAFIRIMSCCK **AAARGRTPPSLGPQDESCTTASSSLA
KDTSS**

(Seq. ID No. 8)

Figure 11

A. Nucleotide sequence of the β 2AR-V2R chimera

atggggcaaccggaacggcagcgccttcttgtctggcaccatagaagccatgcgccggacc
acgacgtcacgcagcaaagggacgaggtgtgggtgggtggcatgggcatcgtcatgtctctcat
cgtcctggccatcgtgtttggcaatgtgctgggtcatcacagccattgccaagttcgagcgtctg
cagacgggtcaccaactacttcatcacttcaactggcctgtgctgatctgggtcatgggcctggcag
tggtgccctttggggccgcccattattcttatgaaaatgtggacttttggcaacttctgggtgcga
gttttggacttccattgatgtgctgtgctgacggccagcattgagaccctgtgcgtgatcgca
gtggatcgctactttgccattacttcaactttcaagtaccagagcctgctgaccaagaataagg
cccggtgatcattctgatgggtgtggattgtgtcaggccttacctccttcttggccattcagat
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(SEQ ID No. 9)

B. Nucleotide sequence of the MOR-V2R chimera

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(SEQ ID No. 10)

C. Nucleotide sequence of the D1AR-V2R chimera

atggctcctaacttctaccatggatgagggcgggctgccagcggagagggatttctcctttc
gcatcctcacggcctgtttcctgtcactgctcatcctgtccactctcctgggcaatacccttgt
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ccccaaagatgagtcctgcaccaccgccagctcctccctggccaaggacacttcatcgtga
(SEQ ID No. 11)

D. Nucleotide sequence of the 5HT1AR-V2R chimera

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(SEQ ID No. 12)

E. Nucleotide sequence of the β 3AR-V2R chimera

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gtcctccttggccaaggacacttcatcgtga
(SEQ ID No. 13)

F. Nucleotide sequence of the Edg1-V2R chimera

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tgggtccccaagatgagtcctgcaccaccgcccagctcctccttggccaaggacacttcatcgtg
a
(SEQ ID No. 14)

FIGURE 12

**β arr2-GFP Translocation to the MOR and MOR-V2R Chimera
in Response to Morphine**

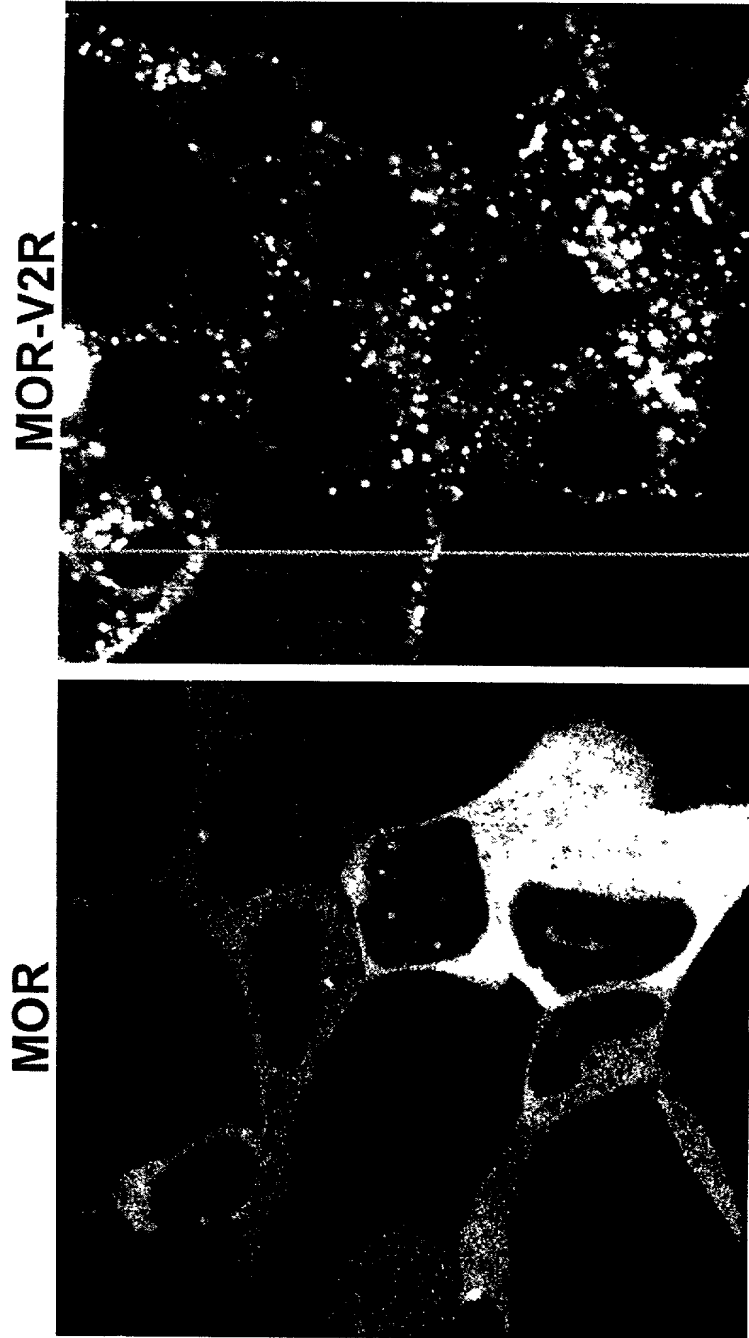


FIGURE 13

**β arr2-GFP Translocation to the D1AR and D1AR-V2R Chimera
in Response to Dopamine**

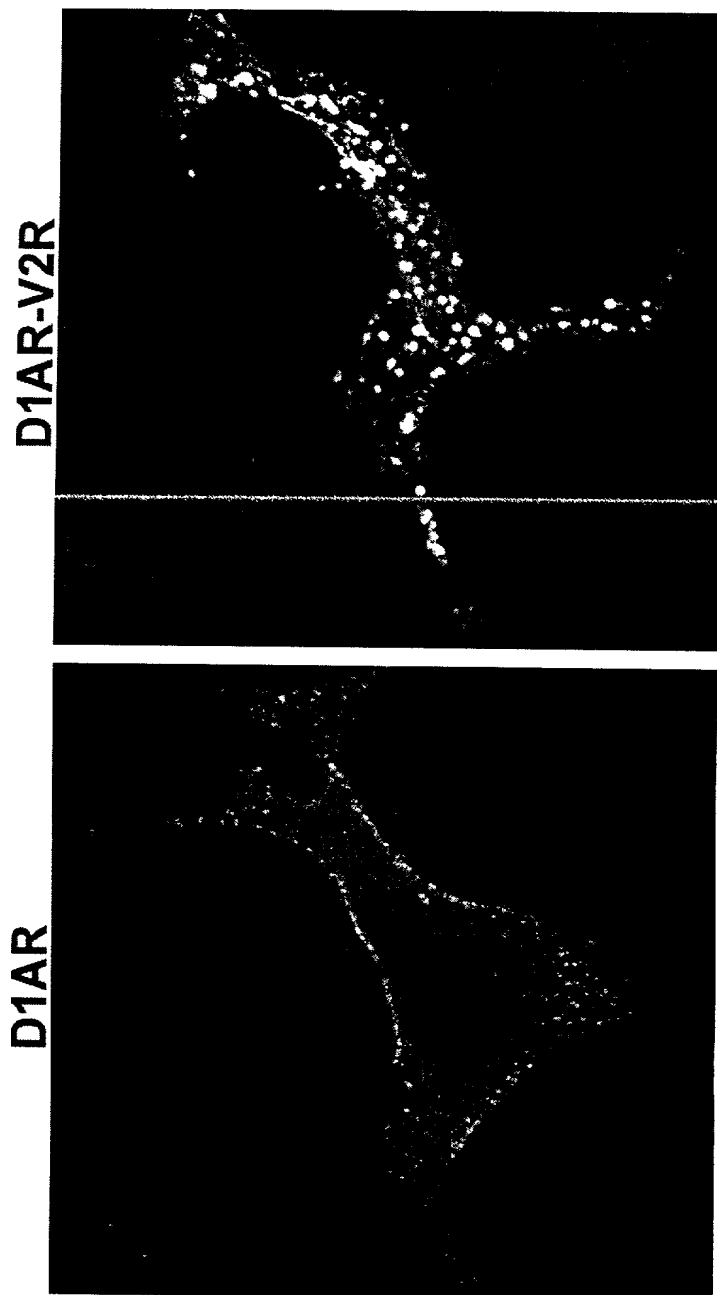


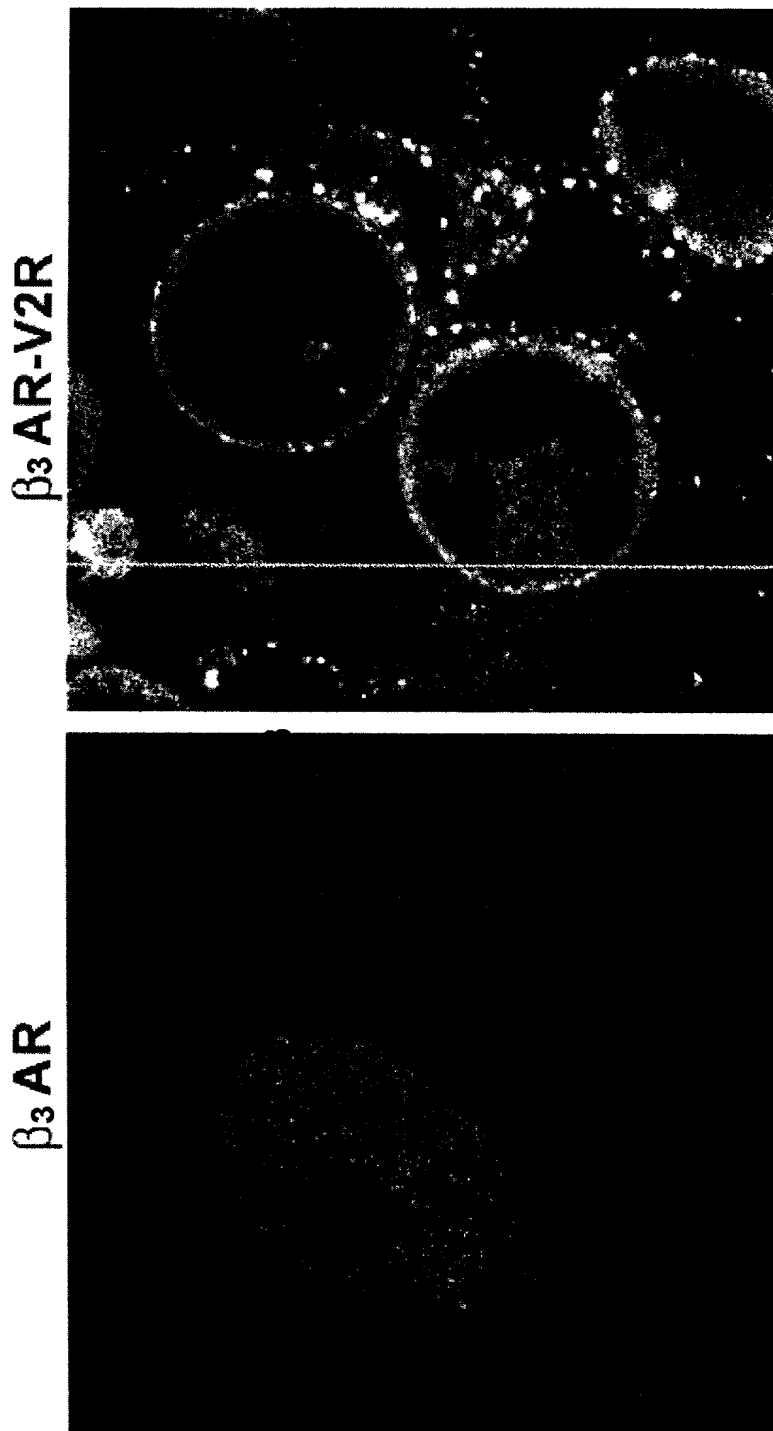
FIGURE 14

β arr2-GFP Translocation to the 5HT1AR and 5HT1AR-V2R
Chimera in Response to Serotonin



FIGURE 15

**β arr2-GFP Translocation to the β_3 AR and β_3 AR-V2R Chimera
in Response to Isoproterenol**



**β arr2-GFP Translocation to the Edg1 and Edg1-V2R Chimera
in Response to Sphingosine-1-Phosphate**

